Eur J Wildl Res
DOI 10.1007/s10344-017-1110-9

ORIGINAL ARTICLE

Conservation in the southern edge of *Tetrao urogallus* distribution: Gene flow despite fragmentation in the stronghold of the Cantabrian capercaillie

Alberto Fameli[1] · **María Morán-Luis**[2,3] · **Rolando Rodríguez-Muñoz**[4] · **María José Bañuelos**[2] · **Mario Quevedo**[3] · **Patricia Mirol**[1]

Received: 2 August 2016 / Revised: 20 April 2017 / Accepted: 1 May 2017

Abstract The Cantabrian capercaillie (*Tetrao urogallus cantabricus*) is an endangered subspecies of the Western capercaillie, endemic of northern Spain, inhabiting the south-western limit of the species range. Assessing genetic variability and the factors that determine it is crucial in order to develop an effective conservation strategy. In this work, non-invasive samples were collected in some of the best preserved areas inhabited by Cantabrian capercaillie. Nine microsatellite loci and a sex-specific marker were analysed. We included five zones, separated by valleys with different levels of habitat modifications. No evidence of genetic clustering was found which suggests that fragmentation and development in the area do not act as barriers to gene flow. Nonetheless, significant differences among sampling zones were encountered in terms of their allelic frequencies (global F_{ST} = 0.035, p = 0.001). Pairwise F_{ST} comparisons showed differences between all sampling zones included, except between the two ones located in the South (Degaña and Alto Sil). These findings, along with the results of individual based genetic differences, indicate that gene flow among sampling zones might be at least slightly compromised, except between the two zones located in the South. Despite this, the sampling zones seem to exchange migrants at a rate that prevents genetic differentiation to the point of creating clusters. Our results show that the capercaillies in the study area constitute a single interbreeding group, which is an important piece of information that provides support to better understand the dynamics of this endangered subspecies.

Keywords Microsatellites · Habitat fragmentation · Gene flow · Genetic structure

Electronic supplementary material The online version of this article (doi:10.1007/s10344-017-1110-9) contains supplementary material, which is available to authorized users.

✉ Alberto Fameli
afameli@macn.gov.ar

[1] Group of Biodiversity and Conservation Genetics, Argentinian Museum of Natural Sciences 'Bernardino Rivadavia', Buenos Aires, Argentina

[2] Research Unit of Biodiversity (UO-PA-CSIC), University of Oviedo, Mieres, Spain

[3] Ecology Unit, Department of Biology of Organisms and Systems, University of Oviedo, Oviedo, Spain

[4] Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Penryn, UK

Introduction

As human modifications of natural habitats increase, more species face stressful environmental conditions such as industrial pollution, pesticides and habitat loss (Bijlsma and Loeschcke 2011). The latter not only affects survival and reproductive rates of individuals but also hampers the evolutionary potential of a species when large populations undergo fragmentation processes, becoming an arrangement of isolated groups with limited gene flow among them. As the level of isolation increases, these smaller groups are more prone to the loss of genetic variability due to genetic drift and inbreeding. Genetic diversity favours adaptation and survival to environmental changes, and there is also a positive correlation between heterozygosity and fitness (Ehrlich 1988; Lande 1988; Ralls et al. 1988; Wayne et al. 1991; Hedrick and Miller 1992; Saccheri et al. 1998; Reed and Frankham 2003; Spielman et al. 2004). Thus, preserving genetic variation is one of the priorities when dealing with conservation of endangered taxa (Frankham and Ralls 1998; Hedrick 2004).

Population subdivision may also occur naturally, especially towards the species' range limit where favourable habitats may be sparse (Lawton 1993; Samis and Eckert 2007).

Published online: 01 June 2017

Therefore, these range-edge populations may show levels of genetic variability that are historically lower than those of core populations, making them more vulnerable to extinction (Lesica and Allendorf 1995; Eckert et al. 2008). Paradoxically, it has been suggested that these populations are of high conservation priority because they may present unique genetic and phenotypic characteristics (Lesica and Allendorf 1995; Ehrlich 1988) given that they usually occupy atypical habitats (Fraser 2000).

The Cantabrian capercaillie (*Tetrao urogallus cantabricus*, Castroviejo 1967) is a peripheral subspecies, endemic of NW Spain. Its distribution is restricted to the Cantabrian Mountains, in the south-western edge of the species' range, separated by more than 300 km from the closest capercaillie population, located in the Pyrenees (González et al. 2010). Recent studies using mitochondrial DNA grouped all capercaillie subspecies into two clearly differentiated lineages named "southern" and "boreal". The Cantabrian population is the only one known to be composed of only "southern" birds. Both lineages contact in the Pyrenees, central and southern Dinaric Alps, Carpathians, Rhodope and Rila Mountains, whereas the rest of the species range is formed by "boreal" birds (Duriez et al. 2007; Rodríguez-Muñoz et al. 2007; Bajc et al. 2011; Klinga et al. 2015). We believe that this phylogeographic differentiation of lineages better reflects the taxonomy of Eurasian capercaillies, instead of the traditional subspecies classification that does not account for the areas where southern and boreal lineages coexist and interbreed.

The Cantabrian capercaillie is classified as Endangered in the Spanish Catalogue of Threatened Species. It also qualifies to be considered as "Endangered" in the Red List of Threatened Species (IUCN), mainly due to rapid population decline, small population size and severely fragmented range (Storch et al. 2006). It has genetic (Rodríguez-Muñoz et al. 2007) and morphological (Castroviejo 1975) traits that set it apart from the rest of the species, being the only population that inhabits pure deciduous forests, a strong contrast with all the other populations which are found in coniferous or mixed forests (Storch 2000; García et al. 2005). These findings suggest that the subspecies may have local adaptations to the atypical environment it inhabits (González et al. 2010). These peculiarities highlight the importance of protecting the Cantabrian capercaillie in order to maintain the adaptive potential of the species, especially in a context of climate change where other populations might face the loss of the coniferous forests they inhabit (Anderson 1991).

Despite this, little is known about the Cantabrian capercaillie in terms of demographic characteristics beyond the generalities of the species. A number of studies have been conducted after capercaillie hunt was banned in Spain (i.e. after 1979), showing a severe decline both in range and abundance (Quevedo et al. 2006; Bañuelos and Quevedo 2008).

Few studies have assessed the status of the Cantabrian capercaillie in terms of genetic variability and the factors that determine its spatial distribution. Alda et al. (2011) and Vázquez et al. (2012) investigated the genetic structure of the subspecies including samples collected along its entire distribution, but these studies reached different conclusions (see "Discussion"). Elucidating these broad scale patterns of genetic diversity is important but so it is assessing fine-scale genetic structure to determine the variables affecting the distribution of genetic diversity at the local level, as well as patterns of gene flow and recolonization of suitable habitat patches. It is likely that habitat management efforts will have an impact at this scale, which means that shedding some light over the processes causing local genetic structure can be useful for designing effective conservation plans. Population dynamics that take place at a small scale are influenced by subtle features, and often their relevance cannot be predicted beforehand. According to Taylor et al. (1993), connectivity among habitat patches is vital for maintaining ecological processes occurring at landscape scale and should be considered along with landscape physiognomy and composition. Relatively low dispersal distances have been reported for other capercaillie populations, and the species is considered practically sedentary (for references, see Storch 1995). Therefore, landscape configuration would be expected to affect capercaillies' gene flow greatly, even at small scale. Nonetheless, sporadic movements covering far greater distances have been recorded (see Storch 1995; Borchtchevski and Moss 2014).

The aim of this work is to explore thoroughly, and at a fine scale, the genetic structure and diversity of the Cantabrian capercaillies inhabiting the western part of the Cantabrian Mountains, a place that has some of the best quality habitats for capercaillies (Quevedo et al. 2006). This scope allows us to make inferences on the factors that might be affecting gene flow and connectivity at an ecological time, precisely in an area considered to be the subspecies stronghold. Here, capercaillies occupy mountain forest patches, separated mainly by a matrix of heather and broom habitats, with low quality areas concentrated at the bottom of the valleys where human activity is more intense. We hypothesised that forest fragmentation and modification by human activities in the area, combined with the polygynous lek-based mating system of capercaillies (del Hoyo et al. 1994), has created a scenario of separated demes with reduced gene flow among them.

Materials and methods

Study area and sampling

The Cantabrian Mountains are located in Northern Spain, running roughly West-East, parallel to the North coast. They create a climatic barrier, Oceanic in the northern exposures and

Continental in the southern exposures. The forests are characterized by a high level of fragmentation, mainly due to historical deforestation by humans (García et al. 2005). Within the Cantabrian area, forests are now mainly restricted to the mountains; therefore, capercaillie habitat is currently confined to altitudes between 800 and 1700 m a.s.l. Within that altitude range, forests are interspersed within a matrix that includes heather and broom areas, meadows, pine plantations, highways, villages and open-pit coal mines.

Our study was conducted in the western part of the Cantabrian Mountains. This area corresponds to the western limit of the subspecies distribution range (del Hoyo et al. 1994). We searched for samples in 62 leks from five zones with known presence of capercaillies: Muniellos Integral Reserve, Hermo, Leitariegos, Degaña and Alto Sil (Fig. 1). These five zones are separated one another by valleys where anthropic modifications reach higher levels (i.e. possible barriers to gene flow). Several roads run through the main valleys that separate Hermo, Degaña, Muniellos and Alto Sil. Deforested areas are especially abundant in the south-facing slopes of the Degaña valley, between the Degaña and Hermo zones, where there is also an open-pit coal mine (Supplementary material, Fig. S1). The minimum linear distance between leks of adjacent sampling zones varied from 3.13 to 4.16 km. The criterion for this division into five zones was made considering that a bird cannot go from one zone to another without crossing a valley.

We collected faeces from leks and surrounding areas, including 71% of all known leks in the study area with occupancy data from 2005 to 2007 (data provided by the Asturian Environmental Agency) during the mating season (April–June) in years 2009 and 2010. Sampling during this period increases the efficiency of detecting individuals while having a lower sampling effort, since the birds tend to congregate around displaying areas. Faeces were stored as documented in Morán-Luis et al. (2014) until DNA extraction. We selected the samples to be included in the study based on their position, size and appearance, in an attempt to maximize the number of different genotyped individuals, following a similar approach than that used by Bellemain et al. (2007). When two faeces had similar size and seemed to have been in the field for a similar period of time, we decided to include both only if the distance between them was 25 m or more.

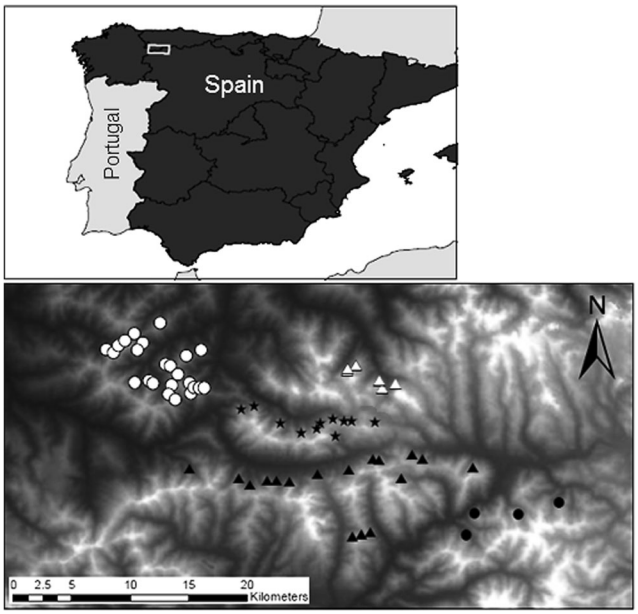

Fig. 1 Study area showing the 62 leks that were visited during the sampling periods. These leks were classified into five different sampling zones: Muniellos (*white circle*), Hermo (*black star*), Leitariegos (*white triangle*), Degaña (*black triangle*) and Alto Sil (*black circle*). *Darker grey* shows lower altitude. Each one of the five sampling zones is separated from the others by low altitude areas. The criterion for this division into zones was made based on the hypothesis that the lower altitude areas (valleys where anthropic modifications are more severe) act as barriers to gene flow

Laboratory procedures

DNA was isolated using QIAamp Stool Mini Kits (QIAGEN) following the manufacturer's instructions with small changes in the protocol (3 ml of ASL buffer and half an InhibitEX tablet per sample). We used one microsatellite locus (TUT1, Segelbacher et al. 2000) to test the success of DNA extractions. When a sample failed to amplify in two independent PCRs, we excluded it from the study. Samples were genotyped using nine microsatellite loci previously developed for *Tetrao urogallus* (TUD2, TUD4, TUD5, TUT1 and TUT3, Segelbacher et al. 2000) and *Tetrao tetrix* (TTD2, TTD6, BG10 and BG15, Caizergues et al. 2001; Piertney and Höglund 2001). Each forward primer was labelled with a fluorescent marker at its 5′ end. PCRs were performed in a final volume of 10 μl containing 1× *Taq* buffer (750 mm Tris-HCl, 200 mM $(NH_4)_2SO_4$, 0.1% (*v*/*v*) Tween 20), 3 mM $MgCl_2$, 0.2 mM of each nucleotide, 4.2 pmol of each primer, 0.108 μg/μl of BSA, 0.335 units of DNA Taq polymerase (Fermentas) and 2 μl of the extracted DNA. PCR programs comprised an initial denaturation of 3 min at 94 °C, 35 cycles of 45 s at 94 °C, 45 s at 54 °C (for BG10 and BG15) or 59 °C (for the rest of the microsatellites) and 45 s at 72 °C. The final extension was carried out at 72 °C for 5 min. A negative control was included in each PCR. The final genotype was obtained from a consensus among several independent PCR replicates. We assigned a heterozygous genotype only after two clear signals obtained independently and a homozygous genotype after three clear signals (modified from Frantz et al. 2003). This resulted in each microsatellite being amplified two to seven times per sample. We genotyped 212 samples collected in 2009 and 192 samples collected in 2010. The genotypes were read independently by three researchers using MegaBACE Fragment Profiler 1.2 software (Amersham Biosciences) and GeneMarker v 1.3 (Soft Genetics LLC). In

case of differences in allele calling by different researchers, a consensus was reached either by repeating the amplification or eliminating that locus for that particular sample. When genotyping samples in multiplexes, it is important to take into account that an intense signal from a fluorescent label in one channel can produce a false signal in another channel (i.e. introgressive signal), therefore leading to incorrect readings. We checked the chromatograms to discard such introgressive signals. We addressed the quality of the samples with the method proposed by Miquel et al. (2006).

Molecular sexing was done based on the amplification of a CHD1 gene intron. This gene is located in the sex chromosomes, and it has different size depending on whether it is the Z or W chromosome variant. We used primers PU and P8mod, designed by Pérez et al. (2011). The forward primer contained a fluorescent label and the products were analysed following the same protocol used for the microsatellites (i.e. minimum of two independent PCRs for heterozygous, three independent PCRs for homozygous), obtaining sex-specific fragments of 192 and 218 bp for the Z and W, respectively.

Summary statistics

We conducted a first round of analyses to identify all different individuals in our sample, using the function Regroup Genotypes in the program GIMLET, version 1.3.3 (Valière 2002). Although this program usually performs well, it can sometimes group together samples that have different genotypes. Besides, the way it displays the genotypes of the samples being grouped together is not easy to read when there are loci with missing data. Therefore, we compared the results obtained with GIMLET with those obtained with the program GENALEX v. 6 (Peakall and Smouse 2006). In our experience, this program is more prone to errors, grouping together samples that are in fact different, but it creates an output that allows to easily compare the samples' genotype and detect such errors. Therefore, combining both programs let us evaluate the accuracy of the grouping performed and correct any errors that could have occurred during that process. We included only those samples that reached a consensus genotype for at least six out of the nine microsatellites. We calculated the probability of identity ($P_{(ID)}$, Paetkau et al. 1995) and the probability of identity among siblings ($P_{(ID)sib}$, Evett and Weir 1998; Waits et al. 2001). Following a conservative approach, we evaluated if our set of nine microsatellites reached a $P_{(ID)sib}$ lower than 0.01 (as proposed by Waits et al. 2001) using the program GIMLET. Given that we also included samples with consensus genotypes in seven or eight loci, we checked whether these combinations of microsatellites were also below the proposed threshold. We discarded the samples with consensus genotypes in only six loci, except for those that had a unique multilocus genotype. Individuals represented by more than one sample were given a spatial position corresponding to the centroid of their samples' distribution.

We calculated deviations from H-W equilibrium proportions for all microsatellites using the program ARLEQUIN version 3.1 (Excoffier et al. 2005). Problems related to the presence of null alleles, large alleles dropout and stuttering were addressed with MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004), using the Bonferroni adjusted 95% confidence interval. Raw allelic richness for each sampling zone was calculated with MICROSATELLITE TOOLKIT (Park 2001), and we also calculated the rarefied allelic richness with the test implemented in HP-RARE 1.0 (Kalinowski 2005), which corrects for the effect of differences in sample size.

Linkage disequilibrium between loci was tested using ARLEQUIN, setting an alpha of 0.01 and correcting it by the Bonferroni method.

We estimated the presence of genotyping errors using the program GIMLET. This procedure was based on 100 samples chosen randomly for each locus.

Temporal vs. spatial variation of allele frequency

Sampling scheme can create strong biases on the inference of population structure (Schwartz and McKelvey 2009). Comparing individuals from different sampling seasons can lead to erroneous conclusions, even when samples are only one year apart (Florin and Höglund 2007). Given that our study includes samples from two consecutive years, we evaluated the possibility of temporal genetic variation overshadowing the spatial variation. Following Florin and Höglund (2007) and Fedy et al. (2008), we computed pairwise F_{ST} comparing data from the same sampling area between the 2 years.

Genetic structure

We computed the inbreeding coefficients (F_{IS}) and population comparisons via pairwise F_{ST} using ARLEQUIN. We also conducted different geographically explicit tests with ALLELES IN SPACE (Miller 2005). As a first attempt to quantify the spatial pattern of genetic variation, we performed an Allelic Aggregation Index Analysis with 10,000 permutations to test the hypothesis of the actual allele distribution being significantly different from the one expected by random distribution, either because of spatial aggregation or uniform distribution. Mantel tests were run for the total group of individuals and for males and females separately, using the same program with 10,000 permutations. A Genetic Landscape Shape Interpolation was also conducted with the same program separately for males, females and the entire group of birds to visualize patterns of genetic diversity and explore the possibility of barriers to gene flow. We used the two

different connectivity networks that can be made with the program: the pairwise location-based network (that compares each individual with all the others) and the Delaunay triangulation-based network (that only compares each individual with the ones around it, see Supplementary material for details). We portrayed the level of genetic difference between pairs of individuals by using their raw genetic distances. The results were overlapped to a map of the area using DIVA-GIS v. 7.5.0 (http://www.diva-gis.org/).

We used two Bayesian assignment approaches to assess the possibility of genetic clustering in our data set. STRUCTURE v. 2.3.4 (Pritchard et al. 2000) was run for $K = 1$ to 15, 10 runs per K, with a burn-in of one million steps followed by three million sweeps. We used an admixture model and correlated allele frequencies, leaving the rest of the parameters in their default setting (inferring alpha and using a lambda value of 1.0). We repeated the analysis, this time inferring lambda as well. The results were summarized with STRUCTURE HARVESTER v. 0.6.94 (Earl and vonHoldt 2012). We assessed the convergence of the different runs performed for the same K value using CLUMPAK (Kopelman et al. 2015). To explicitly address the spatial discontinuities in the distribution of samples, we also used TESS v. 2.3.1 (Chen et al. 2007), which uses geographical coordinates of individuals as priors. All runs with this program were conducted using a spatial interaction parameter of 0.6. First, we ran a test to decide whether our data better fitted the conditional autoregressive (CAR) or Besag, York and Mollié (BYM) admixture model ($K = 2$ to 20, 10 runs per K, 4000 sweeps of burn-in, 24,000 sweeps in total). The CAR model assumes that the fraction of the individuals' genome belonging to a particular cluster is a spatially auto-correlated variable, meaning that individuals that are geographically close to each other are more similar than distant ones. The BYM model (described by Durand et al. 2009) extends the algorithm used in the previous model, including spatial prior distributions on the individual admixture proportions. In other words, the BYM model does not only assume that the individual admixture proportions are spatially auto-correlated but also includes other spatial trends when estimating this variable. Having decided on the best model, we ran it from $K = 2$ to 10, with 10 replicates per K, 51,000 sweeps of burn-in and 300,000 sweeps in total. As a measure of the robustness of the cluster assignment, we calculated the mean confidence assignment of all individuals to their most probable cluster (*q-hat*) as done by Fedy et al. (2008).

Results

Summary statistics

From the 404 genotyped samples, 260 (64%) rendered a number of consensus genotypes enough to be included in the analyses. Two samples had a quality index below 0.62 and were therefore discarded from the analyses. A total of 87 different individuals was identified throughout the study area (Table 1). From the 62 leks inspected, only 39 were represented by at least one individual. The average number of birds per lek was 2.72, and the average number of males per lek was 1.45.

$P_{(ID)SIB}$ was 1.55×10^{-3} for the nine microsatellite set, and it ranged between 2.64×10^{-3} and 7.64×10^{-3} for the different microsatellite configurations in cases where only seven or eight consensus genotypes could be obtained. These values indicate that the real $P_{(ID)}$ was well below the 0.01 threshold proposed by Waits et al. (2001).

The mean number of alleles per locus ranged between 2.778 in Leitariegos and 3.889 in Degaña. The rarefaction method based on a minimum sampling size of six produced similar values of allelic richness for all sampling areas (Table 1).

Loci BG10 and TUT1 showed a significant homozygote excess, while locus TUD4 had heterozygote excess ($\alpha = 0.01$, Table 2). MICRO-CHECKER found evidence of null alleles at loci BG10 and TUT1, the latter also showing evidence of scoring error due to stuttering. Loci TUT3, TUD5, BG15 and TTD2 showed no evidence of errors. Loci TTD6, TUD4 and TUD2 had more than half of their alleles belonging to one size class, so we could not check them for errors.

Linkage disequilibrium was significant for one of the 36 loci pairs (TUT1–BG10, corrected $\alpha = 2.778 \times 10^{-4}$, $p < 1 \times 10^{-5}$). Therefore, we decided to remove locus TUT1 from the samples under analysis. The number of individuals was maintained after this removal ($n = 87$), and the $P_{(ID)SIB}$ for samples with seven or eight consensus genotypes was again below the proposed threshold. Taking this into account, we assumed all samples with the same multilocus genotype as belonging to the same individual. Except for one bird that was detected multiple times in Hermo during 2009 and multiple times in Degaña during 2010, all samples assumed to belong to the same individual were found clustered in the same lek or in adjacent leks. The average number of samples found for each individual was 2.99 (SD = 3.74, Supplementary material, Fig. S2).

The majority of genotyping errors were due to allele dropout, which ranged from 0.006 for TUT1 to 0.108 for TUD4, justifying the need of using a multitube approach to avoid these errors being reflected on the consensus genotype (Table 2).

Temporal vs. spatial variation of allele frequencies

The comparison between years could only be performed for two sampling zones: Degaña (2009: $n = 19$, 2010: $n = 28$) and Muniellos (2009: $n = 15$, 2010: $n = 10$). Pairwise F_{ST} comparisons between these zones and sampling periods resulted in significant values when comparing different zones and non-

Table 1 Capercaillies detected in the study area

Site	*n*						A	
	2009	2010	2009–2010	Males	Females	Undet	Raw	Rar
M	15	10	17	7	9	1	3.444	2.565
H	7	13	15	8	6	1	3.333	2.705
L	4	3	4	3	1	0	2.778	2.655
D	19	28	37	19	17	1	3.889	2.645
AS	13	0	13	8	4	1	3.556	2.649
Total	58	54	87[a]	46[a]	37	4	Mean = 3.400	

M Muniellos, *H* Hermo, *L* Leitariegos, *D* Degaña, *AS* Alto Sil, *n* number of individuals detected from the samples collected either during 2009, 2010 or when pooling together both years, *males* number of males detected when pooling together both years, *females* number of females detected when pooling together both years, *undet* number of birds that could not be sexed when pooling together both years, *A raw* average number of alleles per locus, *A rar* average allelic richness over loci calculated in HP-RARE (Kalinowski 2005)

[a] One male was detected in Hermo during 2009 and in Degaña during 2010. Therefore, we did not assign this individual to any particular sampling zone

significant values when comparing sampling periods for the same zone (Bonferroni corrected α = 0.008, Table 3). Therefore, we pooled together individuals detected in each zone to perform the population structure analyses, regardless of the year in which they were detected.

Spatial genetic structure

To perform the analyses that required assigning the individuals to a particular sampling zone, we excluded the bird detected in two different sampling zones and the four individuals found in Leitariegos, since this sample size was too small to be statistically meaningful. There was no evidence of inbreeding within sampling zones (p value of F_{IS} ranged from 0.593 to 1 for the different sampling zones). Pairwise F_{ST} showed significant differences (Bonferroni corrected α = 0.008) between all sampling zones except for the Degaña-Alto Sil comparison (Table 4).

Allelic Aggregation Index Analysis found a clumped spatial distribution (α = 0.05) in nine unilocus genotypes, from which six were found only in Hermo, Degaña, Alto Sil or combinations of these zones, which corresponds to the center-South part of our study area (Fig. 1). Muniellos Integral Reserve did not have any unique unilocus genotype, despite being well represented in our data set. Mantel test including all the individuals revealed no significant correlation between geographic and genetic distances (p = 0.127). When sexes were included separately, males showed

Table 2 Genetic diversity at the nine loci analysed

Primer	T_a (°C)	*n*	A	H_O	H_E	*p* (HW)	$P_{(ID)}$	$P_{(ID)SIB}$	*D*	F.A.
TUD4	59	77	3	0.779	0.509	<0.001	3.659e-01	5.888e-01	0.108	0.074
BG10	54	81	5	0.407	0.690	<0.001	1.585e-01	4.465e-01	0.042	0.041
TTD6	59	81	4	0.543	0.622	0.027	2.049e-01	4.920e-01	0.039	0.000
TUD2	59	82	3	0.476	0.476	1.000	3.289e-01	5.959e-01	0.072	0.013
TUT1	59	79	5	0.392	0.724	<0.001	1.289e-01	4.223e-01	0.006	0.004
TTD2	59	85	4	0.671	0.651	0.549	1.962e-01	4.753e-01	0.021	0.008
TUT3	59	83	3	0.590	0.627	0.850	2.222e-01	4.941e-01	0.017	0.041
BG15	54	85	5	0.659	0.624	0.663	2.183e-01	4.944e-01	0.028	0.029
TUD5	59	83	6	0.735	0.735	0.673	1.060e-01	4.112e-01	0.067	0.016
Overall			4.222	0.584	0.629		5.082e-07	1.554e-03	0.044	0.025
SE			1.093	0.139	0.089				0.032	0.023

Three microsatellites (TUD4, BG10 and TUT1) showed significant deviations from Hardy-Weinberg equilibrium

T_a optimal annealing temperature, *n* number of individuals successfully genotyped at each locus, *A* observed number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, *p(HW) p* value H-W equilibrium test, $P_{(ID)}$ theoretical expected probability of identity, $P_{(ID)SIB}$ expected probability of identity among siblings, *D* proportion of PCRs showing allele dropout, *F.A.* proportion of PCRs showing false alleles

Table 3 Pairwise F_{ST} comparisons among sampling years for two sampling zones

	M_{2009}	M_{2010}	D_{2009}	D_{2010}
M_{2009}	–	0	0.050	0.044
M_{2010}	0.978	–	0.065	0.063
D_{2009}	**0.003**	**0.005**	–	0
D_{2010}	**0.002**	**0.002**	0.860	–

F_{ST} values are above the diagonal, p values are below the diagonal. Setting an alpha of 0.05, the Bonferroni's adjustment suggests lowering this value to 0.008. Significant p values are shown in bold

M_{2009} Muniellos, 2009 sampling, M_{2010} Muniellos, 2010 sampling, D_{2009} Degaña, 2009 sampling, D_{2010} Degaña, 2010 sampling

significant evidence to consider an isolation by distance pattern ($p = 0.019$), while females showed no such correlation ($p = 0.425$).

The Landscape Shape Interpolation results showed differences when comparing the maps obtained from the two types of connectivity networks (Delaunay triangulation-based vs. pairwise location-based). This indicates that calculating genetic distances by considering only immediate neighbours (Delaunay) does not account for the intricate pattern of genetic relatedness between individuals in the area, where sampling zones could be exchanging migrants. However, some features were congruent between both connectivity networks. In all cases, birds from Muniellos Integral Reserve were separated from the other zones by an area of high levels of genetic differences. Some places inside Degaña also showed high levels of genetic differences between pairs of individuals (Fig. 2).

Contrastingly, birds in Muniellos Integral Reserve were genetically similar, except in the northernmost leks, where females with higher levels of genetic differences were found close to each other. Different patterns were obtained when evaluating males and females separately. Females from Hermo appeared genetically similar to those found in Degaña (Fig. 2c). Males showed a pattern of genetic distances that was somewhat related to the proposed barriers to gene flow (i.e. valleys, Fig. 2b). In Muniellos, Leitariegos and Degaña, males were genetically similar.

STRUCTURE results showed no subdivision of the population under analysis, either when inferring lambda or setting it to a value of 1 (see Fig. 3a, b for results of the runs inferring both alpha and lambda, bar plots shown in Supplementary material, Fig. S3). A high value of Delta K when $K = 2$ can either be interpreted as a high probability of the population being structured in two clusters or a lack of structuring altogether. CLUMPAK showed that all runs performed for the same K converged. Since males showed significant genetic-spatial correlation, we also run STRUCTURE without females. No structure was detected.

Among the TESS runs, the 10 ones with the lowest DIC values were obtained for $K = 5$ (selected admixture model: BYM, Fig. 3c), but the mean *q-hat* indicates that this assignment lacks robustness (Fig. 3d). A closer look to the assignment probability bar plots for $K = 5$ showed none of the individuals as belonging to a particular cluster, therefore suggesting that $K = 1$ is probably reflecting the real scenario (Fig. 3e).

Table 4 Pairwise F_{ST} comparisons among sampling zones, pooling together both years

	M	*H*	*D*	A.S.
M	–	0.044	0.035	0.039
H	**0.007**	–	0.039	0.044
D	**0.003**	**0.002**	–	0.013
A.S.	**0.005**	**0.003**	0.080	–-

F_{ST} values are above the diagonal, p values are below the diagonal. Setting an alpha of 0.05, the Bonferroni's adjustment suggests lowering this value to 0.008. Significant p values are shown in bold. One of the sampling zones (Leitariegos) was not included in the comparisons because only four birds were detected there

M Muniellos, *H* Hermo, *D* Degaña, *A.S.* Alto Sil

Discussion

We found slight differences between sampling zones but no subdivision into genetic clusters. This finding is compatible with a scenario where these zones are connected by gene flow. Previous studies in other parts of Europe have found that significant genetic differentiation can be present in capercaillie populations even when they are only 5–10 km apart from each other (Segelbacher et al. 2003a). In our case, the minimum distance between leks belonging to different sampling zones was roughly the same (Muniellos-Hermo = 3.98 km, Hermo-Degaña = 3.13 km, Degaña-Alto Sil = 3.96 km, Leitariegos-Hermo = 4.16 km).

The lack of genetic structure in our data set could be explained by (1) a general lack of genetic variability in our study area, for example due to a population bottleneck, making it necessary to use more microsatellites to detect genetic subdivision; (2) a restriction to gene flow too recent to be detected by our methodology; or (3) enough levels of gene flow among sampling zones to prevent differentiation.

With the exception of loci TUD4 and TUD2, our sample contained equal or higher number of alleles than those found by Vázquez et al. (2012) for the same microsatellites, even though our study was conducted at a finer scale. The same comment can be made when comparing our results with those found by Segelbacher et al. (2003b), where they assessed the genetic differentiation of capercaillie populations across Europe (although

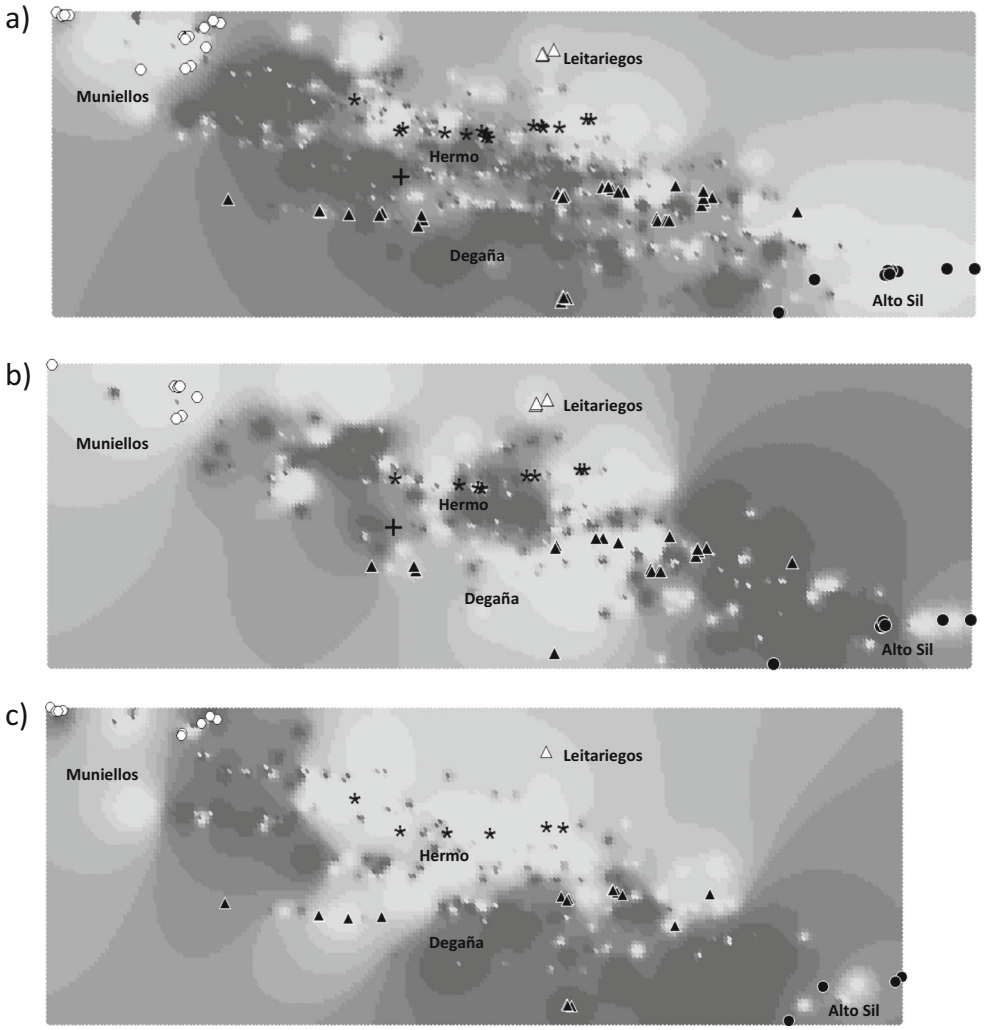

Fig. 2 Landscape Genetic Shape Interpolation obtained from **a** all the individuals, pairwise-based network; **b** males, pairwise-based network; and **c** females, pairwise-based network (the lack of females detected at the Eastern limit of the study area caused this map to be smaller than the others). The *symbols* represent the midpoint assigned to an individual based on its positions, representing the different sampling zones: Muniellos (*white circle*), Hermo (*black star*), Leitariegos (*white triangle*), Degaña (*black triangle*) and Alto Sil (*black circle*). *Light grey*: low levels of genetic differences between individuals. *Dark grey*: high levels of genetic differences between individuals. Some of the individuals overlap at the scale shown and are therefore not evident on the figure

Cantabrian capercaillies were not included in this work). Contrary to what would be expected for a peripheral population, all our sampling zones had an average allelic richness that falls within the range found in that study for other populations. Therefore, we discarded explanation (1) to the lack of genetic structuring. Nonetheless, loci TUT1 and BG10 were linked in our dataset. This is relevant when we take into account that the same was found by Vázquez et al. (2012) for the same subspecies, but to our knowledge, there are no other studies assessing linkage disequilibrium between those two microsatellites in other *T. urogallus* populations. Studies of black grouse (*T. tetrix*) in other parts of Europe have not found such linkage (Höglund et al. 2007; Svobodová et al. 2011; Strand et al. 2012; Segelbacher et al. 2014). Assuming that both species have these microsatellites located on the same chromosomal position, we consider that the linkage in the Cantabrian population could be an evidence of a recent bottleneck event (see Rodríguez-Muñoz et al. 2015). This phenomenon could have caused the loss of allelic combinations for TUT1-BG10 even when total allelic richness was maintained and no genetic structure was created.

Restricted gene flow among sampling zones too recent to leave a mark in the genetic composition is possible, especially when considering anthropic modifications such as open-pit coal mines that occurred during the last 70–50 years. Although we did not find evidence of genetic clustering with STRUCTURE or TESS, four zones did show significant differences in their allelic frequencies when compared with the others, except for the comparison between Degaña and Alto Sil. A closer look to the valley dividing these two zones shows that there are areas where the only gap in forest cover is a road. On the other hand, capercaillies crossing the valley between Degaña and Hermo (the closest adjacent sampling zones) would encounter a more degraded habitat, with a road running along the valley and deforested patches used for agricultural activities and/or having suffered subsequent burning at its sides, especially in the south-facing slopes, together with an open-pit coal mine (Supplementary material, Fig. S1).

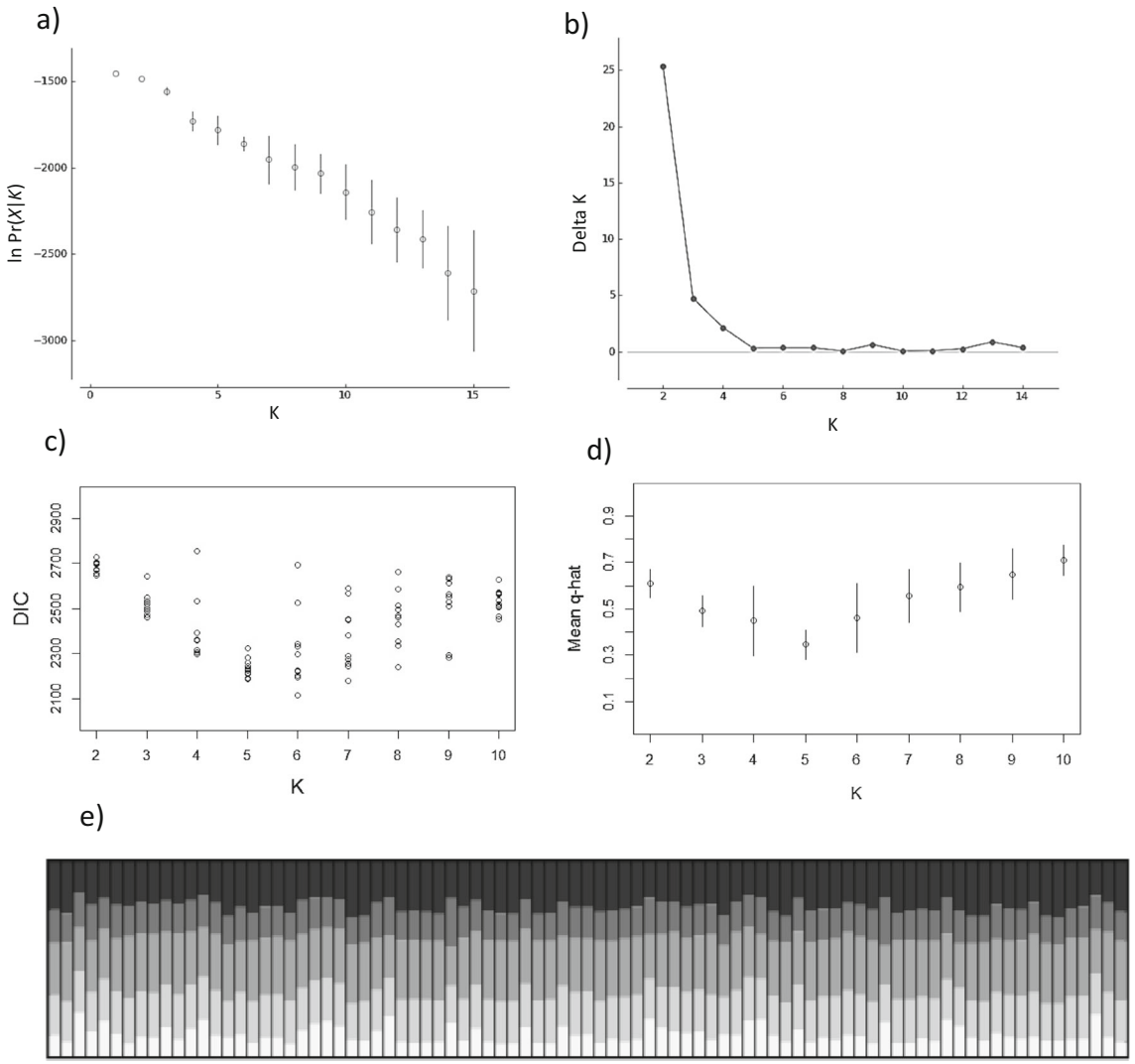

Fig. 3 Graphs used to decide the optimal number of clusters (*K*). **a** Mean log likelihood L(K) (± sd) over 10 STRUCTURE runs for each *K* value from 1 to 15. **b** Evanno's Delta *K* over 10 STRUCTURE runs for each *K* value from 1 to 15. **c** DIC over 10 TESS runs for each *K* value from 2 to 10. **d** Mean confidence assignment of all individuals to their most probable cluster (*q-hat*) over 10 TESS runs for each *K* value from 2 to 10. **e** Bar plot obtained for the best run (i.e. lowest DIC) for *K* = 5 in the clustering analysis performed in TESS. Each bar represents one of the 87 individuals, and the different shades show the estimated proportion of the individual's genotype assigned to each of the *K* = 5 clusters

Movements of birds from one of these zones to the other is likely to be more restricted but definitely possible since we detected a male moving from Hermo to Degaña. Besides, given that the population is small, subdividing it into sampling zones might have a strong sampling effect on their genetic frequencies, resulting in significant differences when comparing them, even if the birds move freely from one zone to another.

Therefore, we conclude that the lack of genetic structuring reflects a real scenario of enough gene flow among sampling zones to maintain connectivity, despite the anthropic modifications in the area. Analysing individual movements and relatedness over time would probably shed some light over this issue.

Alda et al. (2011) conducted a broad scale genetic analysis, including samples collected across the entire extant range of Cantabrian capercaillie. They found evidence to consider three genetic clusters (one in the northern slope of the Cantabrian Mountains, one in the south-eastern slope and one in the south-western slope) with low levels of gene flow between them. We found two problems in their results. First, they found an allelic richness far higher than any other Cantabrian capercaillie study (Rodríguez-Muñoz et al. 2007; Vázquez et al. 2012). Second, two of the three genetic clusters were largely or completely represented by samples collected in a very clumped manner. Taking into account the scale of the study, these genetic groups could be an artefact of the sampling scheme. Vázquez et al. (2012) also investigated the

genetic structure of Cantabrian capercaillies along their entire distribution, using more microsatellites than Alda et al. (2011) but fewer individuals. They found evidence of genetic structure dividing the subspecies in two genetic clusters (one on the West and one on the East), with low gene flow between them. Nonetheless, this work was conducted using linked microsatellites, and therefore, the results should be taken with caution.

We found a differences between males and females regarding their pattern of isolation by distance. Mantel test indicated a slight isolation by distance pattern for males but not for females. This is in accordance with studies in other subspecies (summarized by Storch and Segelbacher 2000) that revealed a higher dispersal capacity by females. The presence of individuals genetically different but spatially close to each other made the Delaunay triangulation unsuitable for assessing the presence of barriers to gene flow (i.e. detail is lost when considering immediate neighbours only). In the case of pairwise distances among all individuals, the genetic interpolation map resembles the findings of the pairwise F_{ST} comparisons between zones: areas with high level of genetic distances were found in the valleys between sampling zones, except for the valley between Degaña and Alto Sil (which also showed non-significant differences for their F_{ST} values).

According to Hampe and Petit (2005), not enough studies have dealt with the low-latitude limit of species ranges, an observation that contrasts with the importance of these range-edge populations (for example, estimates of global change predict greater stability of low-latitude over high-latitude range margins). The authors suggest that conservation strategies should focus more on maintaining as many local, range-edge populations as possible, given their particular genetic structure, instead of focusing on the core populations. This is one of the reasons why the Cantabrian capercaillie is a priority for conservation actions. Management plans will benefit from a thorough knowledge of the distribution of genetic variability and the factors determining it.

Although the Cantabrian subspecies is endangered, this work suggests that current genetic variability and gene flow in our study area are not compromised to the point of requiring specific management actions related to this sort of problems, despite the low population size (Morán-Luis et al. 2014). Knowing that this population inhabiting the so-called subspecies stronghold (Quevedo et al. 2006) is "secure" in terms of genetic variability and gene flow, the research can now be broaden to include other less pristine areas, maybe prioritizing the opposite part of the subspecies range where different alleles are expected to occur. It is important to bear in mind that this work was based in the analysis of neutral markers. A future possibility would be to assess the genetic variability in terms of markers directly related to population viability.

The population in the study area can be treated as a single management unit, with all the implications associated. For example, infectious diseases affecting one sampling zone are expected to create a potential risk to other zones, while leks that go vacant could be naturally claimed by new males.

Due to the complexity of this area (i.e. zones with different conservation status and habitat quality, separated one another by different types of anthropic modifications), we hope the results obtained in this study can serve as a model to understand genetic connectivity and the factors determining it on this endangered subspecies. We also expect the results to be used as part of a sustained genetic monitoring program.

Acknowledgements Many collaborators helped both in the field and with the logistics of the survey. We want to explicitly acknowledge the help of Alberto Fernández-Gil, Beatriz Blanco-Fontao, Elia Palop, Eduardo González, Joaquín Calvo, Carlos Rodríguez del Valle, Damián Ramos, Efrén García and José Carral. Surveying capercaillies in the Cantabrian Mountains required also the sustained cooperation of several rangers of the Asturian Environmental Administration: Fernando Rodríguez Pérez, Antonio Ramos and Antonio González. The environmental authorities of Asturias and Castilla y León granted the permits required to access the display areas in Spring. This study is a contribution to grants IB08-158 (FICYT, Principado de Asturias) to MJ Bañuelos and CGL2010-15990 (MICINN, Spanish Government) to MJ Bañuelos and M Quevedo. We also want to thank María Jimena Gómez Fernández and Fernando Mapelli for their valuable contributions in the interpretation of the results and Duane Thomas Blacklock for his valuable support and assistance.

References

Alda F, Sastre P, De La Cruz-Cardiel PJ, Doadrio I (2011) Population genetics of the endangered Cantabrian capercaillie in northern Spain. Anim Conserv 14:249–260

Anderson JM (1991) The effects of climate change on decomposition processes in grassland and coniferous forests. Ecol Appl 1:326–347

Bajc M, Čas M, Ballian D et al (2011) Genetic differentiation of the Western Capercaillie highlights the importance of South-Eastern Europe for understanding the species phylogeography. PLoS One 6:e23602

Bañuelos MJ, Quevedo M (2008) Update of the situation of the Cantabrian capercaillie *Tetrao urogallus cantabricus*: an ongoing decline. Grouse News 25:5–7

Bellemain E, Nawaz MA, Valentini A, Swenson JE, Taberlet P (2007) Genetic tracking of the brown bear in northern Pakistan and implications for conservation. Biol Conserv 134:537–547

Bijlsma R, Loeschcke V (2011) Genetic erosion impedes adaptive responses to stressful environments. Evol App 5:117–129

Borchtchevski V, Moss R (2014) Age structure of Capercaillie males (*Tetrao urogallus*) in NW Russia may reflect two-way movements—a hypothesis. Ornis Fennica 91:14–28

Caizergues A, Dubois S, Loiseau A, Mondor G, Rasplus JY (2001) Isolation and characterization of microsatellite loci in black grouse (*Tetrao tetrix*). Mol Ecol Notes 1:36–38

Castroviejo J (1967) Eine neue Auerhuhnrasse von der Iberischen Halbinsel. J Ornithol 108:220–221

Castroviejo J (1975) El urogallo en España. Dissertation. Consejo Superior de Investigaciones Científicas, Madrid

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Mol Ecol Notes 7:747–756

Del Hoyo J, Elliot A, Sargatall J (1994) Handbook of the birds of the world. New vultures to guinea fowl. Lynx, Barcelona

Durand E, Jay F, Gaggiotti OE, François O (2009) Spatial inference of admixture proportions and secondary contact zones. Mol Biol Evol 26:1963–1973

Duriez O, Sachet JM, Ménoni E, Pidancier N, Miquel C, Taberlet P (2007) Phylogeography of the capercaillie in Eurasia: what is the conservation status in the Pyrenees and Cantabrian Mounts? Conserv Genet 8:513–526

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Mol Ecol 17:1170–1188

Ehrlich PR (1988) The loss of diversity: causes and consequences. In: Wilson EO (ed) Biodiversity. National Academy Press, Washington DC, pp 21–27

Evett IW, Weir BS (1998) Interpreting DNA evidence: statistical genetics for forensic scientists. Sinauer, Sunderland

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinformatics Online 1:47–50

Fedy BC, Martin K, Ritland C, Young J (2008) Genetic and ecological data provide incongruent interpretations of population structure and dispersal in naturally subdivided populations of white-tailed ptarmigan (*Labapus leucura*). Mol Ecol 17:1905–1917

Florin AB, Höglund J (2007) Absence of population structure of turbot (*Psetta maxima*) in the Baltic Sea. Mol Ecol 16:115–126

Frankham R, Ralls K (1998) Conservation biology: inbreeding leads to extinction. Nature 392:441–442

Frantz AC, Pope LC, Burke TA, Wilson GJ, Delahay RJ, Roper TJ (2003) Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) from faecal DNA. Mol Ecol 12:1649–1661

Fraser DF (2000) Species at the edge: the case for listing of "peripheral" species. In: Darling L (ed) At Risk: Proceedings of a Conference on the Biology and Management of Species and Habitats at Risk. British Columbia Ministry of Environment, Lands and Parks, Victoria, pp 49–53.

García D, Quevedo M, Obeso J, Abajo A (2005) Fragmentation patterns and protection of montane forest in the Cantabrian range (NW Spain). Forest Ecol Manag 208:29–43

González MA, Olea PP, Robles L, Ena V (2010) The Mediterranean *Quercus pyrenaica* oak forest: a new habitat for the Capercaillie? J Ornithol 151:901–906

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters. Ecol Lett 8:461–467

Hedrick PW (2004) Recent developments in conservation genetics. Forest Ecol Manag 197:3–19

Hedrick PW, Miller PS (1992) Conservation genetics: techniques and fundamentals. Ecol Appl 2:30–46

Höglund J, Larsson JK, Jansman HA, Segelbacher G (2007) Genetic variability in European black grouse (*Tetrao tetrix*). Conserv Genet 8:239–243

Kalinowski ST (2005) HP-Rare: a computer program for performing rarefaction on measures of allelic diversity. Mol Ecol Notes 5: 187–189

Klinga P, Mikoláš M, Zhelev P, Höglund J, Paule L (2015) Genetic differentiation of western capercaillie in the Carpathian Mountains: the importance of post glacial expansions and habitat connectivity. Biol J Linn Soc 116:873–889

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15:1179–1191

Lande R (1988) Genetics and demography in biological conservation. Science 241:1455–1460

Lawton JH (1993) Range, population abundance and conservation. Trends Ecol Evol 8:409–413

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? Conserv Biol 9:753–760

Miller MP (2005) Alleles in space: computer software for the joint analysis of interindividual spatial and genetic information. J Hered 96: 722–724

Miquel C, Bellemain E, Poillot C, Bessiere J, Durand A, Taberlet P (2006) Quality indexes to assess the reliability of genotypes in studies using noninvasive sampling and multiple-tube approach. Mol Ecol Notes 6:985–988

Morán-Luis M, Fameli A, Blanco Fontao B et al (2014) Demographic status and genetic tagging of endangered capercaillie in NW Spain. PLoS One 9:e99799

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4: 347–354

Park SJ (2001) The excel microsatellite toolkit. University of Dublin, Dublin

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Pérez T, Vázquez JF, Quirós F, Domínguez A (2011) Improving non-invasive genotyping in capercaillie (*Tetrao urogallus*): redesigning sexing and microsatellite primers to increase efficiency on faeces samples. Conserv Genet Resour 3:483–487

Piertney SB, Höglund J (2001) Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Mol Ecol Notes 1:303–304

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Quevedo M, Bañuelos MJ, Obeso JR (2006) The decline of Cantabrian capercaillie: how much does habitat configuration matter? Biol Conserv 127:190–200

Ralls K, Ballou JD, Templeton A (1988) Estimates of lethal equivalents and the costs of inbreeding in mammals. Conserv Biol 2:185–193

Reed D, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17:230–237

Rodríguez-Muñoz R, Mirol P, Segelbacher G, Fernández A, Tregenza T (2007) Genetic differentiation of an endangered capercaillie (*Tetrao urogallus*) population at the Southern edge of the species range. Conserv Genet 8:659–670

Rodríguez-Muñoz R, Rodríguez del Valle C, Bañuelos MJ, Mirol P (2015) Revealing the consequences of male-biased trophy hunting on the maintenance of genetic variation. Conserv Genet 16:1375–1394

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494

Samis KE, Eckert CG (2007) Testing the abundant center model using range-wide demographic surveys of two coastal dune plants. Ecology 88:1747–1758

Schwartz MK, McKelvey KS (2009) Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. Conserv Genet 10:441–452

Segelbacher G, Paxton RJ, Steinbrück G, Trontelj P, Storch I (2000) Characterization of microsatellites in capercaillie *Tetrao urogallus* (AVES). Mol Ecol 9:1934–1935

Segelbacher G, Storch I, Tomiuk J (2003a) Genetic evidence of capercaillie *Tetrao urogallus* dispersal sources and sinks in the Alps. Wildlife Biol 9:267–273

Segelbacher G, Höglund J, Storch I (2003b) From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. Mol Ecol 12:1773–1780

Segelbacher G, Strand TM, Quintela M et al (2014) Analyses of historical and current populations of black grouse in Central Europe reveal strong effects of genetic drift and loss of genetic diversity. Conserv Genet 15:1183–1195

Spielman D, Brook BW, Briscoe DA, Frankham R (2004) Does inbreeding and loss of genetic diversity decrease disease resistance? Conserv Genet 5:439–448

Storch I (1995) Annual home ranges and spacing patterns of capercaillie in central Europe. J Wildlife Manage 59:392–400

Storch I (2000) Conservation status and threats to grouse worldwide: an overview. Wildlife Biol 6:195–204

Storch I, Segelbacher G (2000) Genetic correlates of spatial population structure in central European capercaillie *Tetrao urogallus* and black grouse *T. tetrix*: a project in progress. Wildlife Biol 6:305–310

Storch I, Bañuelos MJ, Fernández-Gil A, Obeso JR, Quevedo M, Rodríguez-Muñoz R (2006) Subspecies Cantabrian capercaillie *Tetrao urogallus cantabricus* endangered according to IUCN criteria. J Ornithol 147:653–655

Strand TM, Segelbacher G, Quintela M, Xiao L, Axelsson T, Höglund J (2012) Can balancing selection on MHC loci counteract genetic drift in small fragmented populations of black grouse? Ecology and evolution 2:341–353

Svobodová J, Segelbacher G, Höglund J (2011) Genetic variation in Black Grouse populations with different lekking systems in the Czech Republic. J Ornithol 152:37–44

Taylor PD, Fahrig L, Henein K, Merriam G (1993) Connectivity is a vital element of landscape structure. Oikos 68:571–573

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. Mol Ecol Notes 2:377–379

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vázquez JF, Pérez T, Quirós F, Obeso JR, Albornoz J, Domínguez A (2012) Population genetic structure and diversity of the endangered Cantabrian Capercaillie. J Wildlife Manage 76:957–965

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol Ecol 10:249–256

Wayne RK, Lehman N, Girman D et al (1991) Conservation genetics of the endangered Isle Royale gray wolf. Conserv Biol 5:41–51